APPENDIX N BULK MILK TANKER SCREENING TEST FORM

IDEXX - New SNAP® BETA LACTAM

For Raw Bovine Milk

GENERAL REQUIREMENTS

1. See Appendix N General Requirements form items 1-8 & 13 _____

SAMPLES

2. See Appendix N General Requirements (GR) form item 9 _____

APPARATUS & REAGENTS

3. Equipment _____

   a. Heater block with SNAP inset thermostatically controlled at 45±5C _____

      1. Temperature checked by placing standardized thermometer in tube containing liquid (bulb submersed) in heating unit, records maintained _____

      2. Or, use 6 inch partial immersion thermometer placed directly into small thermometer well in middle of heating unit, records maintained _____

   b. Single use 450 µL pipet with indicator line to measure amount of sample, supplied by manufacturer **(screening only)** _____

   c. Fixed volume pipettor to dispense 450 µL (see App. N GR item 7) _____

   d. SNAP Kit _____

      Lot # _____ Exp Date _____ _____

   e. Sample tubes containing reagent pellet _____

   f. Kits received refrigerated _____

   g. Store kits at 0-7C _____

   h. Timer _____

i IDEXX Reader for SNAP devices, with printer or data download capability _____

4. Controls **[FROZEN STANDARDS NOT ALLOWED]** _____

a. Non frozen positive control only, 5.0 ppb Penicillin G _____

b. Store according to label instructions _____

Mfg. _____ Lot # _____ Exp. Date _____ _____

c. Re-hydrate as per manufacturer's instructions _____

d. Test for suitability each time prepared, must produce appropriate reaction, records maintained _____

e. Store control at 0-4.4C for no more than 2 days. **Do not freeze** _____

5. Phosphate Buffer _____

a. Dissolve 2 grams Potassium dibasic phosphate and 8.0 grams of mono-basic potassium phosphate and make up to 1 liter. pH 6.0 ± 0.05 _____

6. Na or K Penicillin G Standard (USP or Human injectable) _____

a. Store according to label instructions _____

Mfg. _____ Lot # _____ Exp. _____ _____

b. Use a 4 or 5 place analytical balance to weigh out the penicillin G _____

c. Calculate the equivalent penicillin G base by using the appropriate correction factor, potency in IU/mg ÷ potency of Pen G^- (1782 IU/mg)(ex. K PenG potency = 1596 IU/mg, purity equal to 1596 ÷ 1782 = 0.895 mg $PenG^-$/mgKPenG) _____

d. Make a 1 mg/mL stock solution by adding drug (100 mg $PenG^-$ ÷ item 6c) (ex. 100 ÷ 0.895 = 111.7 mg KpenG) to a 100 mL volumetric flask and making up with buffer (item 5). May be stored frozen for later dilution. _____

e. Make 1:100 serial dilution of the stock solution, using 100 mL volumetric flask (10 μg/mL stock). May be stored frozen for later dilution. _____

f. Make the final dilution in inhibitor free milk (item 7) to yield the 5.0 ppb standard (ex. 0.5 mL of item 6e. + 999.5 mL milk = 1000 mL of 5 ppb $PenG^{-}$) _____

Date prep. _____ _____

g. Test for suitability each time prepared, must produce appropriate reaction, records maintained _____

h. Store 5.0 ppb control at 0-4.4C for no more than 2 days. **Do not freeze**. _____

7. Inhibitor Free Raw Milk (Negative Control) _____

a. Test for suitability each time prepared, must produce appropriate reaction; records maintained _____

TECHNIQUE

8. Daily Performance and Operation Check (see App. N GR item 10) _____

a. Read positive and negative check set devices. Both devices must read within the limits on the check set devices _____

Positive Range _____ Negative Range _____ _____

If check sets fail, call IDEXX before proceeding _____

9. Test Procedure _____

a. Set out required number of SNAP™ devices, sample tubes and pipets for the samples to be tested. Discard unused, un-refrigerated devices at the end of the day _____

b. Pre-warm heater block(s) to 45±5C, hold at 45C for at least 5 minutes _____

1. Check initial pre-heating with a reference thermometer, records maintained _____

2. Continuous use block heaters, check temperature daily with reference thermometer, records maintained _____

c. Label each device and each sample tube _____

d. Place devices on incubator block(s) _____

e. Mix samples/controls by shaking 25 times in 7 sec through 1 ft arc, use within 3 minutes _____

f. Look for blue reagent pellet in bottom of tube, if not there tap to bring pellet down _____

g. Remove and discard sample tube caps _____

h. With pipets provided, draw up controls or samples (Draw up, avoiding foam and bubbles, expel and draw up again) to the indicator lines and carefully add all of the control or sample milk to the appropriately labeled tubes. (**Screening only)** _____

i. Or, using fixed volume pipettor (item 3c), draw up 450 µL of controls and samples (draw up, avoiding foam and bubbles, expel and draw up again) and carefully add to the appropriately labeled tubes _____

j. Use clean pipet (or tip) for each control and sample _____

k. Agitate sample tube to dissolve reagent pellet _____

l. Incubate tube(s) in heater block next to device with the corresponding ID _____

m. Incubate tubes for 5 minutes (use timer) at 45±5C _____

n. After incubation, pour contents of tubes into sample well of device _____

o. Watch blue activation circle, as it **begins** to become white push the Activator firmly until it "snaps" flush with the body of the SNAP™ device (device remains on heater block) _____

p. Incubate device for 4 minutes (use timer) at 45±5C _____

r. Read **IMMEDIATELY** with IDEXX Reader for SNAP devices _____

10. Interpretation _____

    a. The control spot is on the top and the test spot on the bottom of the Results Window (Correct orientation is with activator button to right and sample well to left) _____

    b. Negative result: _____

        1. If test spot is darker than or equal to the control spot, sample is **Negative (NF)** _____

    c. Positive result: _____

        1. If test spot is lighter than control spot, sample is **Initial Positive** _____

    d. IDEXX Reader for SNAP devices automatically prints results as **Positive** (initial) or **Negative (NF)** _____

11. Confirmation of Initial Positive Samples (see App. N GR item 11) _____

12. Reporting (see App. N GR item 12) _____